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Discordant *Clostridioides difficile* diagnostic assay and treatment practice: a retrospective observational study

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Summary box

Section 1: What is already known on this topic

Diagnosis of *Clostridioides difficile* infection (CDI) relies on multiple-step algorithms that were recently recommended with the aim to avoid CDI overdiagnosis. Since the implementation of this strategy, the proportion of patients with discordant results (positive nucleic acid amplification test [NAAT+]/negative enzyme immunoassay [EIA-]) who receive a treatment for CDI as well as factors influencing the treatment decision remain poorly described.

Section 2: What this study adds

Our study revealed that aapproximately three-quarters of patients treated for *Clostridioides difficile* infection (CDI) who presented discordant test results received a treatment for CDI and almost two-thirds (65%) received a full treatment course of 10 days or more. The treatment decision was associated with the presence of diarrhoea and an abdominal CT scan with signs of colitis. We suggest that the proportion of NAAT+/EIA- patients who received treatment questions the contribution of the EIA for toxin A/B after NAAT to limit overdiagnosis, and additional studies are needed to assess whether other factors are associated with the decision to introduce a treatment in these patients.

Abstract

Objectives: To determine the proportion of treated patients with discordant test results who received a treatment for *Clostridioides difficile* infection (CDI) and to identify patient characteristics associated with the decision to introduce a treatment for CDI.

Design: Retrospective observational study.

Setting: Monocentric study in a Swiss tertiary care hospital.

Participants: Among 4562 adult patients tested for *C. difficile* between March 2017 and March 2019, 239 patients with discordant test results (positive nucleic acid amplification test [NAAT+]/negative enzyme immunoassay [EIA-]) were included.

Main outcome measures: Treatment introduction for CDI.

Results: CDI treatment was introduced in 177/239 (74%) cases. In multivariate analysis, the presence of diarrhoea (adjusted odds ratio (OR) 19.6; 95% confidence interval (CI) 5.3 to 73.2) and an abdominal computed tomography (CT) scan with signs of colitis (OR 7.2; 95% CI 1.6 to 33.5) were independently associated with introduction of treatment. In the symptomatic patient subgroup (n=219), the only factor associated with treatment introduction was an abdominal CT scan with signs of colitis (OR 5.4; 95% CI 1.24 to 23.3).

Conclusions: The proportion of NAAT+/EIA- patients who received treatment questions the contribution of the EIA for the detection of toxin A/B after NAAT to limit overdiagnosis. Additional studies are needed to investigate if other factors are associated with the decision to treat.

Article summary

Strengths and limitations of this study

- This study is one of the few reports concerning the proportion of patients with discordant NAAT/EIA test results that receive specific treatment for C. difficile infection (CDI) since the implementation of multistep diagnostic algorithms according to recently revised guidelines.
- The study results reveal that almost 75% of patients with discordant test results are treated for CDI and this may question the contribution of the EIA for the detection of toxin A/B after NAAT to limit overdiagnosis.
- Among patients with diarrhoea and discordant tests results, the only factor associated with CDI treatment introduction was an abdominal CT scan with signs of colitis.
- Given the monocentric design of this study, our results may reflect local practice only in terms of the diagnostic algorithm and decision to treat.
- The sample size limited the number of variables to investigate, as well as the capacity of the study to detect associations between the investigated factors and the outcome

Introduction

Clostridioides difficile (formerly *Clostridium difficile*) infection (CDI) is a toxin-mediated disease and the leading cause of healthcare-associated infection, as well as an increasing cause of community-associated diarrhoea.[1-4] During the past decade, easy-to perform and low-cost tests for the detection of glutamate dehydrogenase (GDH) and toxins A/B in stool specimens were developed, including a nucleic acid amplification test (NAAT) based on polymerase chain reaction (PCR) and enzyme immunoassays (EIA). However, these tests were not designed as a stand-alone test for CDI diagnosis due to their suboptimal sensitivity and specificity.[5, 6] European and USA guidelines recommend a two- or three-stage diagnostic approach.[5, 7-9] This includes the use of a highly sensitive assay with a high negative predictive value (NPV), either NAAT or EIA for GDH (NPV of 99-100% in a typical endemic situation with a prevalence of 5%) and, if positive, a reflex test using a highly specific confirmatory assay with a high positive predictive value (PPV), typically a toxin A/B EIA (PPV of 98.5%).[5]

CDI diagnosis relies on the association of clinical manifestations and microbiological tests documenting the presence of a toxigenic *C. difficile* strain and toxin/s in stools.[10] Symptomatic patients with both tests positive (NAAT+ or GDH+/EIA+) are likely to suffer from CDI. In the presence of discordant results (NAAT+ or GDH+/ EIA-), the EIA negative result may be interpreted either as a false-negative or a toxin level below threshold in the case of a patient effectively presenting with CDI, or as a true negative in the case of *C. difficile* toxigenic strain carriage. A third-stage test, either NAAT, toxigenic culture or GDH, if not yet performed, can be performed to exclude a false-positive NAAT/GDH,[5, 11] but will not distinguish CDI from toxigenic strain carriage. Therefore, this distinction in patients with discordant results relies on clinical evaluation, but current guidelines do not clearly state which factors should be taken into account.[5, 8]

Although multiple-step algorithms have been recently implemented with the aim to avoid CDI overdiagnosis, it is expected that this two-stage diagnostic strategy should limit treatment prescription in the case of NAAT+/EIA- results. However, it has not been analysed in clinical practice and the actual proportion of patients with discordant results that receive a treatment for CDI remains poorly described, as well as the factors influencing the treatment decision.[12]

In this study, we aimed to identify the proportion of patients that receive a treatment for CDI among those with *C. difficile* discordant test results and patient characteristics associated with the decision to introduce treatment.

Methods

Study design, setting and population

We conducted a retrospective observational study at Geneva University Hospitals, a 2000-bed Swiss tertiary care centre. Clinical and biological data (results of NAAT/EIA assays performed on stool samples) were collected from electronic medical records (EMR) and the hospital bacteriology laboratory, respectively. Inclusion criteria were all adult patients (≥18 years) hospitalised or not, with *C. difficile* toxin assays performed on stool samples between 1 March 2017 and 1 March 2019 that yielded discordant results (NAAT+/EIA-). Exclusion criteria were paediatric patients or those without clinical data available in EMR form. In patients presenting several tests with discordant results, only the first test was considered for analysis. The study was approved by the Geneva cantonal ethics commission and a waiver of informed consent was granted due to its retrospective nature.

Outcomes and definitions

The primary objective was to determine the proportion of adult patients with a first discordant test result who received a treatment for CDI and to identify patient characteristics and risk

factors for CDI (if any) associated with the introduction of treatment. The secondary objective was to determine the patient characteristics and risk factors for CDI associated with the introduction of treatment in a symptomatic subgroup of patients, defined as those presenting with diarrhoea, ileus or toxic megacolon at the time of test prescription.[5]

Treatment for CDI was defined according to the following criteria: an appropriate antibiotic treatment of ≥24 h according to published guidelines,[5, 8, 13] either ongoing or introduced at the time of test prescription, with a written decision in the EMR to start CDI treatment, or without an alternative indication for its prescription. Treatment of 10 days or more was defined as a complete course of treatment. In patients with a previous positive test (NAAT+ or EIA+ or both), only those who had received a treatment for CDI were considered as having a history of CDI. As fecal microbiota transplantation is not performed at our centre, it was not retained in the outcome definition.

Laboratory methods

Since 16 January 2017, the hospital bacteriology laboratory has implemented a two-step diagnostic algorithm comprising the use of a NAAT for *C. difficile* toxin B (*TcdB*; BD MAX™, Becton-Dickinson, Sparks, MD), followed by an EIA for both toxins (A/B; XPect® *C. difficile* Toxin A/B EIA, Remel Inc, San Diego, CA) as a reflex confirmatory test if the NAAT is positive. Fresh stool samples collected in Cairy-Blair tubes are delivered to the laboratory and processed immediately without restrictions related to stool consistency. Samples drawn at night or during the weekend are stored at 4°C in the laboratory before analysis. NAAT and EIA assays are performed daily from Monday to Saturday inclusive.

Statistical analysis

The decision was made to include all eligible patients and no formal sample size calculation was performed. Instead, we restricted the number of investigated parameters before any

confirmatory analysis. Based on the "10 events per variable" rule of thumb, we limited the number of parameters investigated to nine factors selected among known risk factors and clinical characteristics compatible with CDI (see web-only Supplementary Table S1). Patient characteristics and CDI risk factors were described overall and by introduction of a treatment for CDI and reported as frequencies and percentages. A multivariate logistic regression model using a backward stepwise method was performed to determine which parameters were independently associated with the introduction of a CDI treatment. At each step, starting from all nine parameters, the variable with the highest p-value on the likelihood ratio test was removed from the model until all remaining factors were statistically significantly associated with the introduction of CDI treatment at a two-sided level of 5%. The same analysis was performed on a subgroup of symptomatic patients. Missing data were systematically removed from analyses. All statistical analyses were performed using Stata software, version 15 (StataCorp, College Station, TX).

Patient and public involvement

No patients were involved in the design, or conduct, or reporting, or dissemination of our research. The dissemination of the results to the included patients will not be performed.

Results

Patient characteristics

During the study period, 4562 patients had at least one stool sample tested for *C. difficile* (corresponding to 6931 tests). A total of 393 (8.6%) patients (corresponding to 507 tests) had NAAT+ samples; 280/393 (71.3%; corresponding to 352 tests) had an EIA- for toxin A/B testing (NAAT+/EIA-). Among these, 41 (14.6%) were excluded (<18 years [n=33]; without available clinical data in the EMR, apart from demographics [n=8]). Finally, 239 patients (female, 51%) with a first stool sample discordant result were included in the study (Fig. 1).

Baseline patient characteristics are described in Table 1 (baseline characteristics of included patients with NAAT+/EIA-). Since the EIA confirmatory test is a reflex test after a NAAT+, the results of the two tests were available simultaneously in the patient's EMR. Median delay from prescription to results validation was one day (interquartile range (IQR) 0 to 1).

No patient presented with ileus or toxic megacolon, while an alternative diagnosis was reported in the EMR for six patients. Two hundred and nineteen (92%) patients presented with diarrhoea; the remaining 19 did not have symptoms representing an indication for *C. difficile* testing according to published guidelines.[5, 8] Reasons for *C. difficile* testing among the 19 asymptomatic patients are presented in Table 1. One of the six patients who underwent recto-sigmoidoscopy had typical endoscopic lesions.

Introduction of a treatment for CDI

Overall, CDI treatment was introduced in 177 (74%) patients. In univariate analyses, the presence of diarrhoea (odds ratio (OR) 20; 95% confidence interval (CI) 5.6 to 72), an abdominal CT scan with signs of colitis (OR 7.4; 95% CI 1.7 to 32), and a history of CDI (OR 0.44; 95% CI 0.20 to 0.98) were significantly associated with the decision to initiate CDI treatment (Table 2. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in all patients (n=239)). In the backward, stepwise multivariate analysis, the presence of diarrhoea (adjusted OR 19.6; 95% CI 5.3 to 73) and an abdominal CT scan with signs of colitis (adjusted OR 7.2; 95% CI 1.6 to 33) remained independently associated with the initiation of CDI treatment (Table 2).

Treatment type and duration

Twenty-four of 177 patients (13.6%) had already received a treatment active against CDI before validation of the results (Table 3. Treatment type and duration). Among the 177 treated patients, 155 (88%) received a complete course of treatment. In the remaining 22 (12%)

patients with an incomplete CDI treatment, median treatment duration was 7 days (IQR 5 to 9) (Table 3). Of the 175 treated patients with available data regarding severity criteria, 63 (36%) presenting with severity criteria were treated for CDI (metronidazole [41], oral vancomycin [21], and fidaxomicin [1]).

Among the 239 patients included, 35 (15%) had one or more repeated tests with discordant results after the first NAAT+/EIA- (30 [two tests], three [three tests], and two [four tests]) (see web-only Supplementary Fig. S1). Due to the small number in this subgroup (n=35), the association of the above-mentioned variables with CDI treatment introduction was not analysed.

Symptomatic patients

Among the 219 (92%) symptomatic patients, CDI treatment was initiated in 173 (79%) cases. The only variable significantly associated with CDI treatment was an abdominal CT scan with signs of colitis (OR 5.4; 95% CI 1.2 to 23) (Table 4. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in the symptomatic patient subgroup (n=219)).

Discussion

In this study of patients who presented discordant test results (NAAT+/EIA-), approximately three-quarters (74%) received a treatment for CDI and almost two-thirds (65%) received a full treatment course of 10 days or more. These proportions suggest that most patients with discordant test results were considered as having a CDI and treated as such. According to institutional guidelines at the time of study, oral metronidazole was the most frequently administered antibiotic for patients without any severity criteria.[5] Notably, 65% of treated

patients with severity criteria were treated as non-severe CDI. These results highlight issues in treatment decisions in patients with discordant results and severity criteria for CDI.

Results revealed that the presence of symptoms (diarrhoea) and an abdominal CT scan with signs of colitis were significantly associated with the introduction of CDI treatment in NAAT+/EIA- patients (79% of symptomatic patients were treated) and raises the question of the added value of EIA for CDI diagnosis. Regarding the subgroup of symptomatic patients, only an abdominal CT scan with signs of colitis was associated with CDI treatment, a factor known as a convincing clue for active disease.[14, 15]

Our results did not demonstrate any association between a history of CDI and a history of hospitalisation with CDI treatment introduction, despite the known risk of recurrence after a first episode and the risk of CDI and *C. difficile* colonization associated with a history of hospitalization.[16-18] The proportion of treated patients with a history of CDI was lower, but this result was not significant. Concerning the presence of any severity criteria, we did not demonstrate any significant association with the decision to treat, although recent data revealed that leukocytosis and acute renal failure at presentation were associated with poor outcomes in patients with discordant results.[12]

An appropriate indication for CDI testing is key to patient management. The clinical presentation, which ranges from mild diarrhoea (unformed stool) to severe colitis, is a prerequisite for *C. difficile* test prescription in order to avoid overdiagnosis and unnecessary treatment.[19] In this study, 8% of NAAT+/EIA- patients were tested for *C. difficile* in the absence of diarrhoea, ileus or megacolon. Among these, six did not present any indication for *C. difficile* testing according to guidelines, i.e. testing for *C. difficile* carriage and follow-up after CDI treatment,[5, 8] and one was treated for CDI. Although a positive EIA for toxin A/B has been associated with a more severe outcome,[20, 21] data are conflicting regarding the outcomes of patients with NAAT+/EIA- results.[12, 21] When considering the suboptimal

sensitivity of the currently available EIA tests for toxin A/B, clinicians mostly seemed to base their decision to treat patients with discordant results only upon a NAAT+ in order to avoid severe outcomes.

Limitations

This study has several limitations. First, it was monocentric, possibly reflecting local practice only. Second, the sample size limited the number of variables to investigate, as well as the capacity of the study to detect associations between the investigated factors and the outcome. Despite the fact that some are well-known risk factors associated with CDI, few were associated with the decision to treat, which may be due to a lack of power. Third, given the observational design, some covariates may be missing in the model, thus leading to a substantial risk for a phenomenon of confusion. Finally, missing data may have resulted in information bias. Nevertheless, all main clinical characteristics and known risk factors for CDI according to current knowledge were selected for univariate and multivariate analyses.

Recent studies have questioned current algorithms for CDI diagnosis. Pollock et al revealed that the concentration of toxins A, B and A/B tested by single molecule array (Quanterix®, Billerica, MA) were not significantly different in symptomatic (CDI) and asymptomatic (carriage) individuals selected on the basis of a positive NAAT for toxin gene, thus questioning the use of an EIA for toxin A/B after NAAT.[22] By contrast, in patients selected on the basis of a positive toxin test, the concentrations were significantly higher in symptomatic patients, highlighting the need to prioritise toxin detection over toxin gene.[22] Although *C. difficile* toxin gene real-time PCR cycle threshold values cannot be used as a prediction tool in CDI management,[23] the use of a single ultrasensitive assay (Singulex Clarity; Singulex Inc, Alameda, CA) has been shown to be more sensitive and specific compared to a multistep algorithm using NAAT and EIA for toxin A/B.[24]

Regarding the missed opportunity of EIA to avoid overdiagnosis and CDI treatment as revealed by the proportion of treated patients with a negative EIA in our study, similar to Origuen et al,[12] further investigations should be performed to assess the use of ultrasensitive and quantitative immunoassays for toxin A/B detection as stand-alone tests for CDI diagnosis as evoked by recent studies described above.

Conclusions

In conclusion, 5.2% of patients tested for *C. difficile* harboured discordant *C. difficile* test results (NAAT+/EIA-), with 74% receiving a treatment for CDI. The decision to treat was associated with the presence of diarrhoea and an abdominal CT scan with signs of colitis. Nevertheless, additional studies are needed to assess whether other factors are associated with the decision to introduce a treatment in these patients. The proportion of NAAT+/EIA- patients that did not receive any treatment for CDI (26%) questions the contribution of the EIA for the detection toxin A/B after NAAT to limit overdiagnosis.

Data sharing statement

Data may be obtained from a third party and are not publicly available.

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Table legends

Table 1. Baseline characteristics of included patients with NAAT+/EIA-

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record; SD: standard deviation

Table 2. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in all patients (n=239)

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C difficile* infection; EMR: electronic medical record.

Table 3. Treatment type and duration

Abbreviations: CDI: *C. difficile* infection; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay.

Table 4. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in the symptomatic patient subgroup (n=219).

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record

Table 1. Baseline characteristics of included patients with NAAT+/EIA-

	All patients No. (%)		Treatment No. (%)		No treatment No. (%)		p value
	239		**177**	**(74.1)**	**62**	**(25.9)**	
Age, mean (SD)	66.8	(18.7)	67.4	(18.7)	64.9	(19.1)	0.367
Age ≥ 65 years old[1]	154	(64.4)	117	(66.1)	37	(59.7)	0.363
Gender, female n (%)	122	(51.1)	93	(52.5)	29	(46.8)	0.434
Hospitalisation[1], n (%)	213	(89.1)	162	(91.5)	51	(82.3)	**0.044**
- Internal medicine	112	(46.9)	82	(46.3)	30	(48.4)	
- Surgery	45	(18.8)	33	(18.6)	12	(19.4)	
- Intensive care unit	6	(2.5)	5	(2.8)	1	(1.6)	
- Emergency	19	(8)	16	(9)	3	(4.8)	
- Rehabilitation	13	(5.4)	13	(7.3)	-		
- Oncology and hematology	16	(6.7)	12	(6.8)	4	(6.5)	
- Gynaecology and obstetrics	2	(0.8)	1	(0.6)	1	(1.6)	
Outpatients	26	(10.9)	15	(8.5)	11	(17.7)	
Presence of symptoms[1]							
- Diarrhoea[2]	219/238	(92)	173/176	(98.3)	46	(74.2)	**0.000**
- Ileus	-	-	-	-	-	-	
- Toxic megacolon	-	-	-	-	-	-	
- Presence of an alternative diagnosis in EMR	6/219	(2.7)	3/176	(1.7)	3	(4.8)	
Absence of symptoms[1] (diarrhoea, ileus or toxic megacolon)	19/238	(8)	3/176	(1.7)	16	(25.8)	
Reasons for testing in asymptomatic patients:							
- Abdominal pain of unknown origin	5	(26.3)	-		5	(31.3)	
- Change in stool consistency[3]	6	(31.6)	1	(33.3)	5	(31.3)	
- Testing for carriage	3	(15.8)	-		3	(18.8)	
- Follow-up after treatment for CDI	3	(15.8)	1	(33.3)	2	(12.5)	
- No justification	2	(10.5)	1	(33.3)	1	(6.3)	
Any severity criteria[1,4]	79/236	(33.5)	63/175	(36)	16/61	(26.2)	0.164
Complicated[1,5]	6/236	(2.5)	6/175	(3.4)	-	-	0.143
- Sepsis	4	(1.7)					
- Hypotension	1	(0.4)					
- Septic shock	1	(0.4)					
Body mass index ≥ 30[1]	33/231	(14.3)	27/171	(15.8)	6/60	(10)	0.270
Creatinine clearance ≤ 60ml/min[1]	86/236	(36.4)	67/176	(38.1)	19/60	(31.7)	0.374
Immunosuppression[1,6]	51	(21.3)	37	(20.9)	14	(22.6)	0.782
Abdominal imaging (CT)	88	(36.8)	67	(37.9)	21	(33.9)	0.576
- Radiologic signs of colitis	37/88	(42	35	(19.8)	2	(3.2)	**0.002**
Ongoing PPI treatment[1]	138/236	(58.5)	105/176	(59.7)	33/60	(55)	0.527
History of hospitalisation[1,7]	227	(95.0)	168	(94.9)	59	(95.2)	0.939
History of CDI[1,8]	29	(12.1)	17	(9.6)	12	(19.4)	**0.043**
History of antibiotic treatment[1,9]	158	(66.1)	118	(66.7)	40	(64.5)	0.758
Infectious disease specialist advice[10], n (%)	77	(32.2)	59	(33.3)	18	(29)	0.533

[1]At the time of testing.
[2]≥ 3 unformed stools in 24 h.
[3]Not corresponding to the definition of diarrhoea
[4]Blood leucocytes >15 G/l or serum creatinine > 133 µmol/L.
[5]Ileus, toxic megacolon, septic shock or hypotension.
[6]Including chemotherapy ≤60 days before test prescription; SOT; HSCT; steroid (minimum 20 mg/d prednisone or equivalent during at least 4 weeks before test prescription).
[7]Any hospitalization of ≥ 48 h in the last 12 weeks before test prescription
[8] History of positive test results in EMR (NAAT +/EIA+ or EIA + or TC +)
[9]Any antibiotic treatment of ≥ 48 h in the last 4 weeks before test prescription
[10]Any recommendation about treatment

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record; SD: standard deviation

Table 2. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in all patients (n=239)

	Likelihood of receiving treatment for CDI OR (95% CI)								
Characteristics	**Treatment n=177 (74.1%)**		**No treatment n=62 (25.9%)**		**Unadjusted**		**p-value**	**Adjusted**	**p-value**
Age ≥ 65 years old	117	(66.1)	37	(59.7)	1.31	(0.73 – 2.38)	0.366		
Presence of diarrhoea[1]	173/176	(98.3)	46	(74.2)	20.1	(5.6 – 71.8)	**0.000**	19.6 (5.3–73.2)	**0.000**
Any severity criteria[2]	63/175	(36)	16/61	(26.2)	1.6	(0.83 – 3.03)	0.166		
Immunosuppression[3]	37	(20.9)	14	(22.6)	0.91	(0.45 – 1.82)	0.782		
Radiologic signs of colitis	35	(19.8)	2	(3.2)	7.4	(1.7 – 31.7)	**0.007**	7.2 (1.6–33.5)	**0.012**
Ongoing PPI treatment	105/176	(59.7)	33/60	(55)	1.2	(0.67 – 2.2)	0.527		
History of hospitalisation[4]	168	(94.9)	59	(95.2)	0.95	(0.25 – 3.62)	0.939		
History of CDI[5]	17	(9.6)	12	(19.4)	0.44	(0.2 – 0.98)	**0.047**		
History of antibiotic treatment[6]	118	(66.7)	40	(64.5)	1.1	(0.6 – 2.02)	0.758		

[1]≥3 unformed stools in 24 h.
[2] Blood leucocytes count >15 G/l or serum creatinine >133 µmol/L.
[3] Including chemotherapy ≤ 60 days before test prescription; SOT; HSCT; steroid (minimum 20 mg/d prednisone or equivalent during at least 4 weeks before test prescription).
[4] Any hospitalisation of ≥48 h in the last 12 weeks before test prescription.
[5] History of positive test results in EMR (NAAT +/EIA+ or EIA + or TC +).
[6] Any antibiotic treatment of ≥48 h in the last 4 weeks before test prescription.

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C difficile* infection; EMR: electronic medical record.

Table 3. Treatment type and duration

		No. (%)
CDI treatment, n (%)	**177**	
- Metronidazole (oral)	154	(87)
- Metronidazole (intravenous)	1	(0.6)
- Vancomycin (oral)	21	(11.9)
- Fidaxomicin (oral)	1	(0.6)
Patients with a complete treatment course (≥10 days), n (%)	155	(87.6)
Patients with an incomplete treatment course (<10 days), n (%)	21	(11.9)
- Infectious disease specialist advice	6	(28.6)
- Alternative diagnosis	2	(9.5)
- Death	5	(23.8)
- Unknown	8	(38.1)
Median duration of treatment (all), days (IQR)	11	(10–15)
- Complete course of treatment	11	(11–15)
- Incomplete course of treatment	7	(5–8)
Timing of CDI treatment introduction, n (%)		
- Difference between time of test prescription and results < 24h	52	(29.4)
o Ongoing treatment at the moment of test prescription	3	
o CDI treatment introduced after NAAT+/EIA- results	49	
- Difference between time of test prescription and results >24 h	125	(70.6)
o Ongoing treatment at the moment of test prescription	10	
o CDI treatment introduction at the time of test prescription	11	
o CDI treatment introduction at the time of NAAT+/EIA- results	104	

Abbreviations: CDI: *C. difficile* infection; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay

Table 4. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment introduction in the symptomatic patient subgroup (n=219).

	Likelihood of receiving treatment for CDI OR (95% CI)								
Characteristics	**Treatment n= 173 (79%)**		**No treatment n= 46 (21%)**		**Unadjusted**		**p value**	**Adjusted**	**p value**
Age ≥ 65 years old	114	(65.9)	29	(63)	1.13	(0.57 – 2.22)	0.718		
Any severity criteria[2]	60/171	(35.1)	13/45	(28.9)	1.33	(0.64 – 2.72)	0.435		
Immunosuppression[3]	37	(21.4)	9	(19.6)	1.11	(0.49 – 2.52)	0.788		
Radiologic signs of colitis	34	(19.7)	2	(4.4)	5.4	(1.24 – 23.3)	**0.024**	5.4 (1.24 – 23.3)	**0.024**
Ongoing PPI treatment	104/172	(60.5)	23	(50)	1.5	(0.79 – 2.94)	0.203		
History of hospitalisation[4]	164	(94.8)	43	(93.5)	1.3	(0.32 – 4.9)	0.939		
History of CDI[5]	16	(9.3)	7	(15.2)	0.56	(0.22 – 1.48)	0.245		
History of antibiotic treatment[6]	117	(67.6)	30	(65.2)	1.11	(0.56 – 2.21)	0.757		

[1]≥3 unformed stools in 24 h.
[2] Blood leucocytes count >15 G/l or serum creatinine >133 µmol/L.
[3] Including chemotherapy ≤ 60 days before test prescription; SOT; HSCT; steroid (minimum 20mg/d prednisone or equivalent during at least 4 weeks before test prescription).
[4] Any hospitalisation of ≥48 h in the last 12 weeks before test prescription.
[5] History of positive test results in EMR (NAAT +/EIA+ or EIA + or TC +).
[6] Any antibiotic treatment of ≥48 h in the last 4 weeks before test prescription.

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record

Figure legends

Figure 1. Flowchart of patient selection.

Abbreviations: NAAT: nucleic acid amplification test for toxin B; EIA: enzyme immunoassay for toxin A/B; EMR: electronic medical records.

Supplementary data

Supplementary Table S1. Definitions of patient characteristics selected for univariate and multivariate analysis

Abbreviations: CDI: *C. difficile* infection; WBC: white blood count; NAAT: nucleic acid amplification test for toxin B gene; EIA: enzyme immunoassay for toxin A/B; TC: toxigenic culture; PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant.

Supplementary Figure S1. Patients (n=35) with repeated tests with discordant results (n=77).

Abbreviations: T: treated to first test; U: untreated to first test; TT: treated to first and second tests; TU: treated to first test, untreated to second test; UT: untreated to first test, treated to second test; UU: untreated to first and second tests; TTT: treated to first, second and third tests; TUT: treated to first test, untreated to second test and treated to third test.

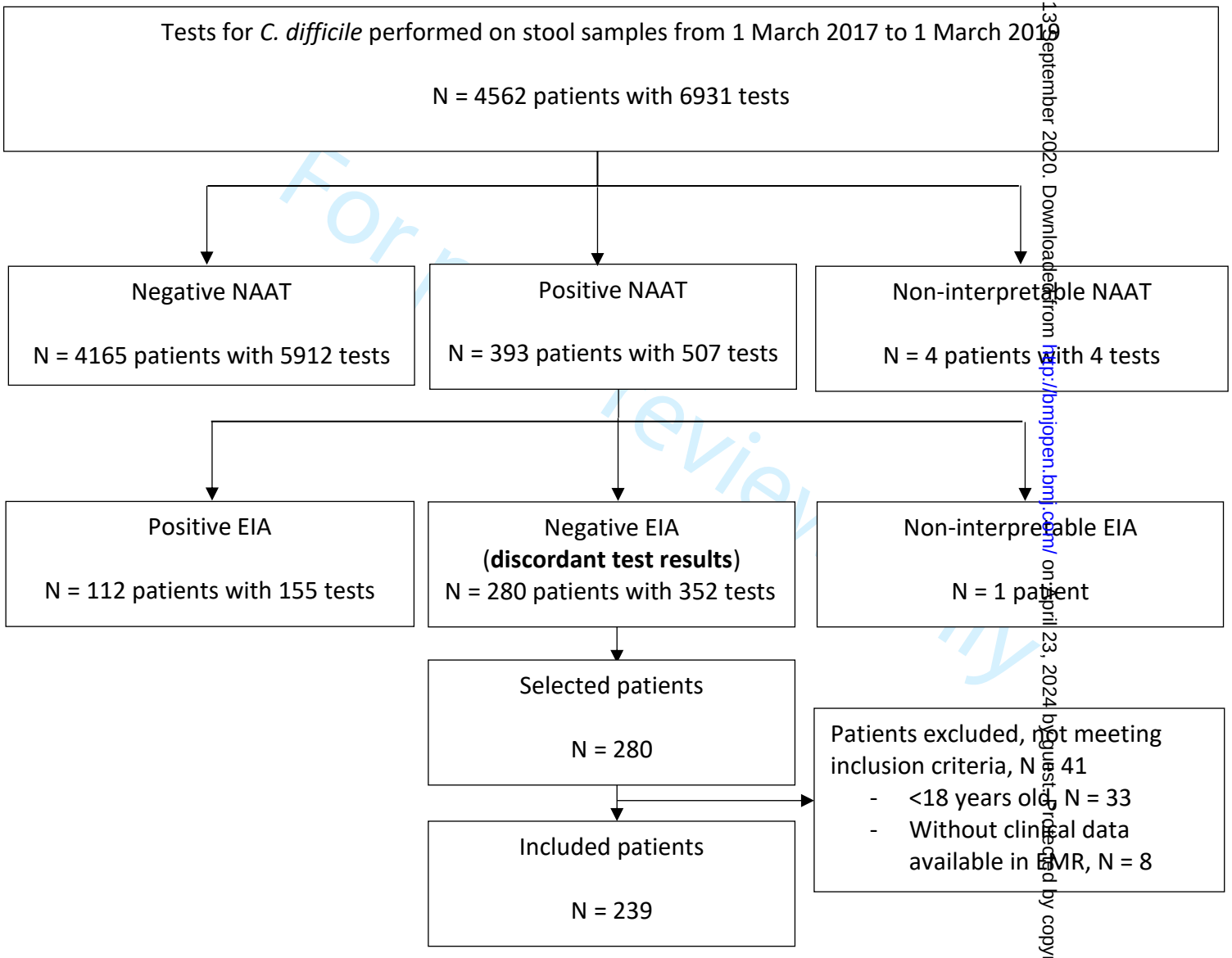

Table S1. Definitions of patient characteristics selected for univariate and multivariate analysis.

Data	Definitions
Demographic data	Age (≥65 years*), gender, patient location when tested for CDI (wards if hospitalised, outpatients)
Diarrhoea *	≥ 3 unformed stools in ≤24hours[1]
Severity criteria *	WBC >15 G/l or serum creatinine > 133 µmol/L[2]
Radiologic sign of colitis *	Abdominal computed tomography (CT) scanner with signs of colitis[3]
Obesity	Body mass index ≥ 30[4]
Chronic renal insufficiency	Creatinine clearance < 60ml/min[5]
History of hospitalisation *	≥ 48 h ≤12weeks before prescription[6]
History of CDI *	All patients with a history of positive test results (NAAT+ or EIA+ or TC+)[7]
History of antibiotic treatment *	≥ 48 h ≤4 weeks before prescription[8]
Ongoing PPI treatment *	Any ongoing PPI treatment at the moment of the prescription[9]
Immunosuppression *	Chemotherapy ≤60 days before prescription; SOT, HSCT, steroid**[10-13]
Treatment course for CDI	
- Complete	≥10 days
- Incomplete	<10 days

* Patient characteristics selected for univariate and multivariate analysis
** At least 20 mg/d (prednisone or equivalent) during ≥4 weeks before prescription

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Figure S1

	Second test - treated	Second test - untreated	Total
T	11	16	27
U	2	6	8
Total	13	22	35

	Third test - treated	Third test - untreated	Total
TT	1	1	2
TU	0	0	0
UT	0	0	0
UU	0	1	1
Total	1	2	3

	Fourth test - treated	Fourth test - untreated	Total
TTT	1	0	1
TUT	1	0	1
Total	2	0	2

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6-8
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	8-9
Study size	10	Explain how the study size was arrived at	8-9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	9
		(c) Consider use of a flow diagram	9
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10 (table 1)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10-11
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12-13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Discordant Clostridioides difficile diagnostic assay and treatment practice: a cross-sectional study in a tertiary care hospital, Geneva, Switzerland

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Discordant *Clostridioides difficile* diagnostic assay and treatment practice: a cross-sectional study in a tertiary care hospital, Geneva, Switzerland

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Transparency: The manuscript's guarantors (LL, MCZ and JS) affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Abstract

Objectives: To determine the proportion of patients who received a treatment for *Clostridioides difficile* infection (CDI) among those presenting a discordant *Clostridioides difficile* diagnostic assay and to identify patient characteristics associated with the decision to treat CDI.

Design: Cross-sectional study.

Setting: Monocentric study in a tertiary care hospital, Geneva, Switzerland.

Participants: Among 4562 adult patients tested for *C. difficile* between March 2017 and March 2019, 208 patients with discordant tests' results (positive nucleic acid amplification test [NAAT+]/negative enzyme immunoassay [EIA-]) were included.

Main outcome measures: Treatment for CDI.

Results: CDI treatment was administered in 147 (71%) cases. In multivariate analysis, an abdominal computed tomography scan with signs of colitis (OR 14.7; 95% CI 1.96-110.8) was the only factor associated with CDI treatment.

Conclusions: The proportion of NAAT+/EIA- patients who received treatment questions the contribution of the EIA for the detection of toxin A/B after NAAT to limit overtreatment. Additional studies are needed to investigate if other factors are associated with the decision to treat.

Article summary

Strengths and limitations of this study

- Patients were considered as treated for *C. difficile* infection according to pre-defined criteria, including the appropriateness of the antibiotic treatment for *C. difficile* infection, timing of its introduction and duration, and the absence of any alternative justification for its prescription.
- Parameters investigated in multivariate analysis were limited to a selection of risk factors and clinical characteristics known to be associated with *C. difficile* infection.
- Patients without an indication for *C. difficile* testing were excluded from the study.
- Given the monocentric design of the study, our results may reflect local practice only in terms of the diagnostic algorithm and decision to treat.
- Given the observational design of the study and the routinely-collected origin of the data, some covariates may be missing in the model, thus leading to a risk for a phenomenon of confusion.

Introduction

Clostridioides difficile (formerly *Clostridium difficile*) infection (CDI) is a toxin-mediated disease and the leading cause of healthcare-associated infection, as well as an increasing cause of community-associated diarrhoea.[1-4] During the past decade, easy-to-perform and low-cost diagnostic tests have been developed, comprising nucleic acid amplification tests (NAAT) for the detection of toxin A/B genes and enzyme immunoassays (EIA) for the detection of glutamate dehydrogenase (GDH) and toxins A/B in stool specimens. However, these tests are not recommended as stand-alone tests for CDI diagnosis due to their suboptimal sensitivity and specificity.[5 6] European and USA guidelines recommend a two- or three-stage diagnostic approach.[5 7-9] This includes the use of a highly sensitive assay with a high negative predictive value (NPV), either NAAT or EIA for GDH (NPV of 99-100% in a typical endemic situation with a prevalence of 5%) and, if positive, a reflex test using a highly specific confirmatory assay with a high positive predictive value (PPV), typically a toxin A/B EIA (PPV of 98.5%).[5]

CDI diagnosis relies on the association of clinical manifestations and microbiological tests documenting the presence of a toxigenic *C. difficile* strain and toxin/s in stools.[10] Symptomatic patients with both tests positive (NAAT+ or GDH+/EIA+) are likely to suffer from CDI. In the presence of discordant results (NAAT+ or GDH+/ EIA-), the EIA negative result may be interpreted either as a false-negative or a toxin level below threshold in the case of a patient effectively presenting with CDI, or as a true negative in the case of *C. difficile* toxigenic strain carriage. A third-stage test, either NAAT, toxigenic culture or GDH, if not yet performed, can be performed to exclude a false-positive NAAT/GDH,[5 11] but will not distinguish CDI from toxigenic strain carriage. Therefore, this distinction in patients with discordant results relies on clinical evaluation, but current guidelines do not clearly state which factors should be taken into account.[5 8]

CDI overdiagnosis and subsequent overtreatment are major concerns regarding the emergence of resistance, particularly vancomycin-resistant *Enterococcus spp*.[12] Although multiple-step algorithms have been recently implemented with the aim to avoid CDI overdiagnosis and subsequent overtreatment, the actual proportion of NAAT+/EIA- patients who receive a treatment for CDI remains poorly described, as well as the factors influencing the treatment decision.[13]

In this study, we aimed to identify the proportion of patients that receive a treatment for CDI among those with *C. difficile* discordant tests' results (NAAT+/EIA-) and patient characteristics associated with the decision to treat.

Methods

Study design, setting and population

We conducted a cross-sectional study at Geneva University Hospitals, a 2000-bed Swiss tertiary care centre. Clinical and biological data (results of NAAT/EIA assays performed on stool samples) were collected from electronic medical records (EMR) and the hospital bacteriology laboratory, respectively. Inclusion criteria were all adult patients (≥18 years) hospitalised or not, with *C. difficile* toxin assays performed on stool samples between 1 March 2017 and 1 March 2019 that yielded discordant results (NAAT+/EIA-). Exclusion criteria were asymptomatic patients (without diarrhoea, ileus or toxic megacolon), paediatric patients, patients with a treatment against *C. difficile* introduced ≥ 48 h before the results of tests, or without clinical data available in EMR form. In patients presenting several tests with discordant results over the study period, only the first test was considered for analysis. The study was approved by the Geneva cantonal ethics commission and a waiver of informed consent was granted due to its retrospective nature.

Outcomes and definitions

The primary objective was to determine the proportion of adult patients with a first discordant test result who received a treatment for CDI and to identify patient characteristics and risk factors for CDI (if any) associated with CDI treatment.[5]

Patients were considered as treated for CDI if they fulfilled all of the following criteria: 1) an appropriate antibiotic treatment administered for CDI according to published guidelines[5 8 14]; 2) treatment introduced less than 48 h before the results of tests; 3) treatment duration of ≥10 days or still under treatment at time of death; and 4) treatment prescribed with a written decision in the EMR for CDI treatment, or without an alternative indication for its prescription. Of note, as fecal microbiota transplantation is not performed at our centre, it was not retained in the outcome definition.

In patients with a previous positive test (NAAT+ or EIA+ or both), only those who had received a treatment for CDI were considered as having a history of CDI. Abdominal computed tomography (CT) scans were considered if they were performed less than 48 h before and less than 10 days after the test result. Definitions of other characteristics and risks factors are described in the web-only supplementary table S1.

Laboratory methods

Since 16 January 2017, the hospital bacteriology laboratory has implemented a two-step diagnostic algorithm comprising the use of a NAAT for *C. difficile* toxin B (*TcdB*; BD MAX™, Becton-Dickinson, Sparks, MD), followed by an EIA for both toxins (A/B; XPect® *C. difficile* Toxin A/B EIA, Remel Inc, San Diego, CA) as a reflex confirmatory test if the NAAT is positive. Fresh stool samples collected in Cairy-Blair tubes are delivered to the laboratory and processed immediately without restrictions related to stool consistency. Samples drawn at night or during the weekend are stored at 4°C in the laboratory before analysis. NAAT and EIA assays are performed daily from Monday to Saturday inclusive.

Statistical analysis

The decision was made to include all eligible patients and no formal sample size calculation was performed. Instead, we restricted the number of investigated parameters before any confirmatory analysis. Based on the "10 events per variable" rule of thumb, we limited the number of parameters investigated to eight factors selected among known risk factors and clinical characteristics compatible with CDI. Patient characteristics and CDI risk factors were described overall and by treatment for CDI and reported as frequencies and percentages. A multivariate logistic regression model using a backward stepwise method was performed to determine which parameters were independently associated with CDI treatment. At each step, starting from all eight parameters, the variable with the highest p-value on the likelihood ratio test was removed from the model until all remaining factors were statistically significantly associated with CDI treatment at a two-sided level of 5%. Sensitivity analyses were performed to assess the robustness of the results when deceased patients were a) excluded from the analysis and b) considered as not treated. Missing data were systematically removed from analyses. Statistical significance was assessed at a two-sided 0.05 level for all analyses. All statistical analyses were performed using Stata software, version 15 (StataCorp, College Station, TX).

Patient and public involvement

No patients were involved in the design, or conduct, or reporting, or dissemination of our research. The dissemination of the results to the included patients will not be performed.

Results

Patient characteristics

During the study period, 4562 patients had at least one stool sample tested for *C. difficile* (corresponding to 6931 tests). A total of 393 (8.6%) patients (corresponding to 507 tests) had NAAT+ samples; 280/393 (71.3%; corresponding to 352 tests) had an EIA- for toxin A/B testing (NAAT+/EIA-). Two hundred and eighty (6.1%) patients had 352 (5.1%) discordant test results (figure 1). Among these, 72 (25.7%) were excluded (<18 years [n=33]; asymptomatic patients [n=20]; without available clinical data in the EMR, apart from demographics [n=9]; with treatment against *C. difficile* introduced 48 h or more before the results of tests [n=10)]). We hereby analysed the first NAAT+/EIA- stool sample of the 208 patients included in the study (figure 1). Baseline patient characteristics are described in Table 1 (table 1. Baseline characteristics of included patients with NAAT+/EIA- (n=208)). Since the EIA confirmatory test is a reflex test after a NAAT+, the results of the two tests were available simultaneously in the patient's EMR. Median delay from prescription to results validation was one day (interquartile range (IQR) 0 to 1).

Among the 208 patients included, none presented with ileus or toxic megacolon, while an alternative diagnosis was reported in the EMR for six patients. One of five patients who underwent recto-sigmoidoscopy had typical endoscopic lesions and was treated. Fifty-nine patients (28%) had an abdominal CT scan and 49 received a treatment for CDI (table 1). A CT scan was performed before the tests' results in 15/59 (25%) patients and after results in 44 patients. The most frequent indications for the CT scan were: investigation for an abdominal infection (40%); signs of colitis (32%); and urological disease (12%). Among patients with signs of colitis, a CT scan was performed to investigate CDI in 16 (53%) patients.

Treatment, treatment type and duration

Overall, 147 patients (71%) were treated for CDI. Treatment consisted of oral metronidazole for 132 patients (90%) and oral vancomycin for 15 patients (10%) (table 2. Treatment type and duration). Treatment was initiated at the time of test results in 133 patients (90%) and

within the 48 h preceding the results in the remaining 14. Of the 145 treated patients with available data regarding severity criteria, 55 (38 %) presenting with severity criteria were treated for CDI (oral metronidazole [n=46], oral vancomycin [n=9]). Among untreated patients (n=61), 46 (75%) did not receive any CDI treatment and 15 (25%) received a treatment for CDI during less than 10 days (median duration of treatment, 7 days; IQR, 4.5-8.5).

Associated factors

In univariate and multivariate analyses, abdominal CT scan with signs of colitis was the only associated factor with CDI treatment (OR 14.7; 95% CI 1.96-110.8) (table 3: Univariate and multivariate regression models for the association of patients characteristics with CDI treatment (n=208)).

Discussion

In this study of patients who presented discordant test results (NAAT+/EIA-), 71% received a treatment for CDI, suggesting that most patients with discordant test results were considered as having a CDI and treated as such. These findings raise the question of the added value of EIA for CDI diagnosis. According to institutional guidelines at the time of the study, oral metronidazole was the most frequently administered antibiotic for patients without any severity criteria.[5] Notably, 84% of treated patients with severity criteria were treated as non-severe CDI and these results highlight issues in treatment decisions in patients with discordant results and severity criteria for CDI. Results revealed that an abdominal CT scan with signs of colitis was significantly associated with CDI treatment in NAAT+/EIA- patients. Indeed, radiological signs of colitis are known as a convincing clue for active disease.[15 16]

We did not demonstrate any association between a history of CDI and a past hospitalisation with CDI treatment. The proportion of patients with a history of CDI was lower among

treated patients, but this result was not significant. These findings were surprising considering the risk of CDI recurrence after a previous CDI, and the risk of CDI associated with a history of hospitalisation.[17-19] Concerning the presence of any severity criteria, we did not demonstrate any significant association with the decision to treat, although recent data revealed that leukocytosis and acute renal failure at presentation were associated with poor outcomes in patients with discordant results.[13]

Although a positive EIA for toxin A/B has been associated with a more severe outcome,[20 21] data are conflicting regarding the outcomes of patients with NAAT+/EIA- results.[13 21] When considering the suboptimal sensitivity of the currently available EIA tests for toxin A/B, clinicians mostly seemed to base their decision to treat patients with discordant results only upon a NAAT+ in order to avoid severe outcomes.

Limitations

This study has several limitations. First, it was monocentric, possibly reflecting local practice only. Second, the sample size limited the number of variables to investigate, as well as the capacity of the study to detect associations between the investigated factors and the outcome. Despite the fact that some are well-known risk factors associated with CDI, few were associated with the decision to treat, which may be due to a lack of power. Third, given the observational design, some covariates may be missing in the model, thus leading to a substantial risk for a phenomenon of confusion. Missing data may have resulted in information bias. Nevertheless, all main clinical characteristics and known risk factors for CDI according to current knowledge were selected for univariate and multivariate analyses. Finally, one of the most important factors in the decision to treat that could not be analysed in the present study is human behaviour, which depends on the clinician's experience and each individual clinical situation.

Recent studies have questioned current algorithms for CDI diagnosis. Pollock et al revealed that the concentration of toxins A, B and A/B tested by a single molecule array were not significantly different in symptomatic (CDI) and asymptomatic (carriage) individuals selected on the basis of a positive NAAT for toxin gene, thus questioning the use of an EIA for toxin A/B after NAAT.[22] By contrast, in patients selected on the basis of a positive toxin test, the concentrations were significantly higher in symptomatic patients, highlighting the possibility to prioritise toxin detection over toxin gene.[22] *C. difficile* toxin gene real-time PCR cycle threshold (CT) values have been associated in some studies with toxin-EIA positive results and adverse outcomes. However, data are conflicting and the accuracy of CT values for toxin-positive prediction remains low with currently available EIA assays.[23] The use of a single ultrasensitive assay has been shown to be more sensitive and specific compared to a multistep algorithm using NAAT and EIA for toxin A/B.[24]

Regarding the missed opportunity of EIA to avoid overdiagnosis and CDI treatment as revealed by the proportion of treated patients with a negative EIA in our study, similar to Origuen et al,[13] further investigations should be performed to assess the use of ultrasensitive and quantitative immunoassays for toxin A/B detection as stand-alone tests for CDI diagnosis as evoked by recent studies described above.

Conclusions

In conclusion, 5.2% of patients tested for *C. difficile* harboured discordant *C. difficile* test results (NAAT+/EIA-), with 71% receiving a treatment for CDI. An abdominal CT scan with signs of colitis was the only factor associated with the decision to treat. Nevertheless, additional studies are needed to assess whether other factors are associated with the decision to treat these patients. The proportion of NAAT+/EIA- patients that did not receive any

treatment for CDI (29%) questions the contribution of the EIA for the detection toxin A/B after NAAT to limit CDI overdiagnosis and overtreatment.

Data sharing statement

Extra data can be accessed via the Dryad data repository at http://datadryad.org/ with the doi: 10.5061/dryad.jm63xsj7r

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Table 1. Baseline characteristics of included patients with NAAT+/EIA- (n=208)

	All patients No. (%)	Treatment No. (%)	No treatment No. (%)	p value
	208	**147 (71)**	**61(29)**	
Age, mean (SD)	66 (19)	67 (19)	64 (20)	0.309
Age ≥ 65 years old[1]	133 (64)	93 (63)	66 (30)	0.752
Gender, female n (%)	104 (50)	72 (49)	32 (52)	0.648
Hospitalisation[1], n (%)	186 (89)	134 (91)	52 (85)	0.207
- Internal medicine	97 (47)	67 (46)	30 (49)	
- Surgery	39 (19)	25 (17)	14 (23)	
- Intensive care unit	5 (2)	4 (3)	1 (2)	
- Emergency	17 (8)	15 (10)	2 (3)	
- Rehabilitation	13 (6)	13 (9)	0	
- Oncology and haematology	13 (6)	9 (6)	4 (7)	
- Gynaecology and obstetrics	2 (1)	1 (1)	1 (2)	

Table legends

Table 1. Baseline characteristics of included patients with NAAT+/EIA- (n=208)

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record; SD: standard deviation

Table 2. Treatment type and duration

Abbreviations: CDI: *C. difficile* infection; IQR: interquartile range

Table 3. Univariate and multivariate regression models for the association of patients' characteristics with CDI treatment (n=208)

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C difficile* infection; EMR: electronic medical record.

Symptoms[1]				
- Diarrhoea[2]	208	147 (100)	61 (100)	
- Ileus				
- Toxic megacolon				
Presence of an alternative diagnosis in EMR	6 (3)	1 (1)	5 (8)	**0.009**
Any severity criteria[1,3]	72/205 (35)	55/145 (38)	17/60 (28)	0.190
Complicated[1,4]	6/205 (3)	5/145 (3)	1/60 (2)	0.673
- Sepsis	4 (2)	4 (3)	0	
- Hypotension	1 (0.5)	1 (1)	0	
- Septic shock	1 (0.5)	0	1 (2)	
Body mass index ≥ 30[1]	29/200 (15)	21/142 (15)	8/58 (14)	0.856
Creatinine clearance ≤ 60ml/min[1]	74/205 (36)	54/146 (37)	20/59 (34)	0.677
Immunosuppression[1,5]	44 (21)	31 (21)	13 (21)	0.971
Abdominal imaging (CT)	59 (28)	49 (33)	10 (16)	**0.014**
- Radiologic signs of colitis	30 (14)	29 (20)	1 (2)	**0.001**
Ongoing PPI treatment[1]	119/207 (57)	84/146 (58)	35 (57)	0.983
History of hospitalisation[1,6]	196 (94)	139 (95)	57 (93)	0.750
History of CDI[1,7]	19 (9)	12 (8)	7 (11)	0.450
History of antibiotic treatment[1,8]	137 (66)	96 (65)	41 (67)	0.792
Infectious disease specialist advice[9], n (%)	64 (31)	43 (29)	21 (34)	0.462

[1]At the time of testing.
[2]≥ 3 unformed stools in 24 h.
[3]Blood leucocytes >15 G/l or serum creatinine > 133 µmol/L.
[4]Ileus, toxic megacolon, septic shock or hypotension.
[5]Including chemotherapy ≤60 days before test prescription; SOT; HSCT; steroid (minimum 20 mg/d prednisone or equivalent during at least 4 weeks before test prescription).
[6]Any hospitalisation of ≥ 48 h in the last 12 weeks before test prescription
[7] History of positive test results in EMR (NAAT +/EIA+ or EIA + or TC +)
[8]Any antibiotic treatment of≥ 48 h in the last 4 weeks before test prescription
[9]Any recommendation about treatment

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record; SD: standard deviation

Table 2. Treatment type and duration

	No. (%)	
CDI treatment, n (%)	**147**	**(70.7)**
- **Metronidazole (oral)**	132	(89.8)
- **Vancomycin (oral)**	15	(10.2)
Median duration of treatment, days (IQR)	**11**	**(11 – 15)**
Timing of CDI treatment introduction		
- **Treatment introduced ≤ 48 h prior to test results**	14	(9.5)
- **Treatment introduced at the time of test results**	133	(90.5)

Abbreviations: CDI: *C. difficile* infection ; IQR : interquartile range

Table 3. Univariate and multivariate regression models for the association of patient characteristics with CDI treatment (n=208)

	Likelihood of receiving treatment for CDI OR (95% CI)								
	Treatment n= 147 (70.7%)		No treatment n= 61 (29.3%)		Unadjusted		p value	Adjusted	p value
Characteristics									
Age ≥ 65 years	93	(63.3)	40	(65.6)	0.9	(0.48 – 1.69)	0.752		
Any severity criteria[2]	55/145	(37.9)	17/60	(28.3)	1.54	(0.8 – 2.97)	0.192		
Immunosuppression[3]	31	(21.1)	13	(21.3)	0.98	(0.47 – 2.04)	0.971		
Radiologic signs of colitis	29	(19.7)	1	(1.6)	**14.7**	**(1.96 – 110.8)**	**0.009**	**14.7 (1.96 – 110.8)**	**0.009**
Ongoing PPI treatment	84/146	(57.5)	35	(57.4)	1	(0.54 – 1.84)	0.983		
History of hospitalisation[4]	139	(94.6)	57	(93.4)	1.21	(0.35 – 4.2)	0.754		
History of CDI[5]	12	(8.2)	7	(11.5)	0.68	(0.25 – 1.83)	0.452		
History of antibiotic treatment[6]	96	(65.3)	41	(67.2)	0.91	(0.48 – 1.72)	0.792		

[1]≥3 unformed stools in 24 h.
[2] Blood leucocytes count >15 G/l or serum creatinine >133 µmol/L.
[3] Including chemotherapy ≤ 60 days before test prescription; SOT; HSCT; steroid (minimum 20mg/d prednisone or equivalent during at least 4 weeks before test prescription).
[4] Any hospitalisation of ≥48 h in the last 12 weeks before test prescription.
[5] History of positive test results in EMR (NAAT +/EIA+ or EIA + or TC +).
[6] Any antibiotic treatment of ≥48 h in the last 4 weeks before test prescription.

Abbreviations: PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant; NAAT: nucleic acid amplification test; EIA: enzyme immunoassay; TC: toxigenic culture; CDI: *C. difficile* infection; EMR: electronic medical record

Figure legends

Figure 1. Flowchart of patient selection.

Abbreviations: NAAT: nucleic acid amplification test for toxin B; EIA: enzyme immunoassay for toxin A/B; EMR: electronic medical records.

Supplementary data

Supplementary Table S1. Definitions of patient characteristics selected for univariate and multivariate analysis

Abbreviations: CDI: *C. difficile* infection; WBC: white blood count; NAAT: nucleic acid amplification test for toxin B gene; EIA: enzyme immunoassay for toxin A/B; TC: toxigenic culture; PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant.

Figure 1.

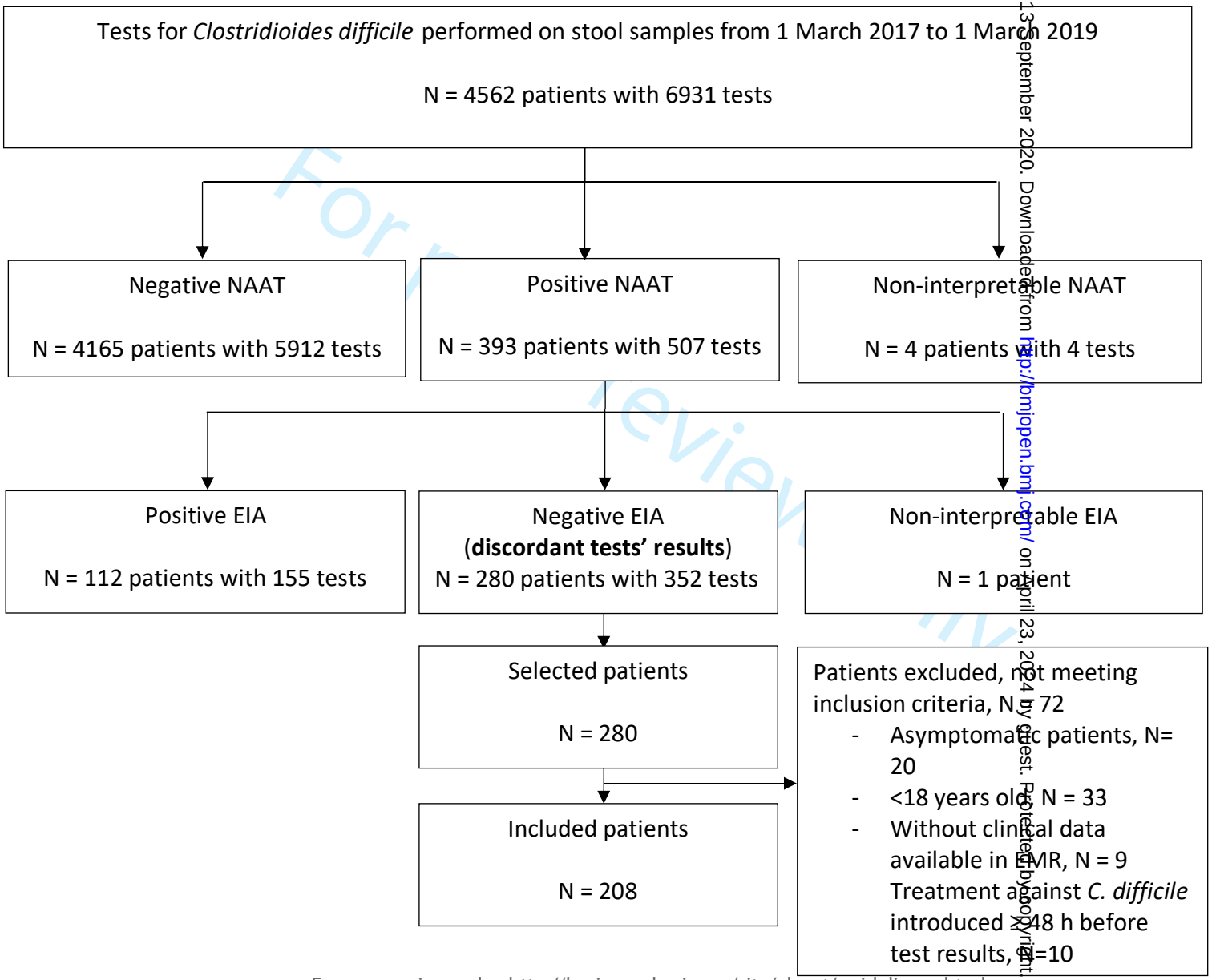

Table 1. Definitions of patient characteristics

Data	Definitions
Demographic data	Age (≥65 years*)
Severity criteria *	WBC >15 G/l or serum creatinine > 133 µmol/L [1]
Radiologic sign of colitis *	Abdominal computed tomography (CT) scanner with signs of colitis [2] performed < 48 h before and < 10 days after test result
Obesity	Body mass index ≥ 30 [3]
Chronic renal insufficiency	Creatinine clearance < 60ml/min [4]
History of hospitalisation *	≥ 48 h ≤ 12weeks before prescription [5]
History of CDI *	All patients with a history of positive test results (NAAT+ or EIA+ or TC+) who had received a treatment for CDI [6]
History of antibiotic treatment *	≥ 48 h ≤ 4 weeks before prescription [7]
Ongoing PPI treatment *	Any ongoing PPI treatment at the moment of the prescription [8]
Immunosuppression *	Chemotherapy ≤60 days before prescription; SOT, HSCT, steroid[1] [9-12]
Treatment course for CDI	Introduced < 48 h before test results with a duration of ≥ 10 days[2]

* Patient characteristics selected for univariate and multivariate analysis
[1] At least 20 mg/d (prednisone or equivalent) during ≥4 weeks before prescription
[2] Or still under treatment at time of death

Abbreviations: CDI: *C. difficile* infection; WBC: white blood count; NAAT: nucleic acid amplification test for toxin B gene; EIA: enzyme immunoassay for toxin A/B; TC: toxigenic culture; PPI: proton pump inhibitor; SOT: solid organ transplant; HSCT: hematopoietic stem cell transplant.

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6 - 7
Objectives	3	State specific objectives, including any prespecified hypotheses	7
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	7 – 8
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7 – 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7 – 8, 9
Bias	9	Describe any efforts to address potential sources of bias	12
Study size	10	Explain how the study size was arrived at	9
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	9

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	10 – 11
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11, 20
		(*b*) Report category boundaries when continuous variables were categorized	10 – 11, 18
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	13 – 14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.